UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF CHEMISTRY

ISOLATION AND CHARACTERIZATION OF THE MAJOR COMPONENT OF THE LEAF OIL OF *EUCALYPTUS GLOBULES* PLANT

A THESIS SUBMITTED TO DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

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ABSTRACT

The aim of this study was to isolate and characterize the major component of the leaf oil of *Eucalyptus globules* plant. Accordingly the extraction was performed by Hydrodistillation method and major component isolated. The structure of major component was characterized by spectroscopic methods including IR, ^{1}H NMR, ^{13}C NMR and DEPT. The major component was found to be 1, 3, 3–Trimethyl-2-Oxabicyclo [2.2.2]–octane.

1. INTRODUCTION

Natural products are organic compounds that are formed by living systems. The elucidation of their structures and chemistry, synthesis and biosynthesis are major areas of organic chemistry. Naturally occurring compounds may be occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids, the common amino acids and sugars. They are known as primary metabolites.

Compounds that are characteristic of a limited range of species are secondary metabolites. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites.[1]

Natural products have been investigated and utilized to alleviate disease since early human history. In the early 1900s, before the ‘‘Synthetic Era’’, 80% of all medicines were obtained from roots, barks and leaves. Their dominant role is evident in the approximately 60% of anticancer compounds and 75% of drugs for infectious diseases that are either natural products or natural product derivatives.

Some of the opportunities for natural products discovery and development are in pharmaceuticals, agrochemicals, cosmetics, chemicals and pharmaceutical agents. From 1981 to 2002 reveals that natural products continued to their roles. More than 90% of current therapeutic classes derive from a natural product prototype and interestingly, even today roughly two-thirds to three quarters of the world’s population relies upon medicinal plants for its primary pharmaceutical care.

Natural products have pointed the way to the future. Many significant advances in science and industry have been inspired by the pursuit of capturing the value of natural products. Efforts toward total synthesis of natural products have resulted in development of new synthetic methods, advances in the fields of medicinal chemistry, and other aspects of the science of drug development and, of course with provision of a continuous and reliable supply of new drugs to

The pharmaceutical industry.[2] Natural products (secondary metabolites) have been the most successful source of potential drug leads. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest.

The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh) which are still used today to treat coughs, colds and inflammation. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases.[3]

For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin, digitoxin, morphine, quinine, and pilocarpine. All of these compounds, or derivatives thereof, are still in use as drugs today.[4]

In folk medicine, medicinal herbs and plant products were commonly used for the treatment of infectious disease, in treating a wide spectrum of infections and other diseases. Today, a great number of different medicinal tea and other plant products are available in market (including cosmetics and pharmaceuticals), which contains biologically active substances. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant derived drugs have been reported to be safe and without side effects.

A survey of literature reveals that there are many essential oils which possess antifungal activity. In tuberculosis, treatment contains high doses of antibiotic due to resistance and side effects of this antibiotic, patients take more time for cure. Therefore, we need to search plant derived antifungal drugs which are safe and without side-effects. Hence, it is of interest to determine the scientific basis for the traditional use medicinal plants.[5] Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also

Often with adulterations and side effects. Therefore, the need to search for plants of medicinal value.[6] Eucalyptus is known to be a rich source of secondary compounds with a variety of biological activities. The use of Eucalyptus oils, mostly obtained from the leaves, is grouped as medicinal, industrial, aromatic and flavoring, depending on their chemical composition.

Eucalyptus oil is sold world-wide. Most of the oil sold in Europe and North America is used in products such as Vicks Vaporub. Medicinal Eucalyptus oil produced from *Eucalyptus globules* is widely used for the relief of cold and influenza symptoms. It is a unique natural product having antiseptic properties and the power to clear the nasal passages and bronchial tubes making it easier to breathe. A popular new use is to vaporize it in saunas. It is an excellent rub for muscular aches and pains and it has been widely used for many years by sportsmen to help keep muscles trim and supple.

A use which is gaining widespread acceptance is the practice of adding Eucalyptus oil to the laundry wash for cleaning and freshening clothes, which utilizes its cleaning, deodorizing and antiseptic properties. Medicinal Eucalyptus oil is used extensively as a raw material and active ingredient in cough lozenges, inhalation sprays and drops, gargles, mouth washes, toothpastes, balms and ointments, liniments and soaps. Eucalyptus oil is also used in antiseptics and germicidal disinfectants because of its pleasant odor and its effectiveness in killing bacteria.[7] *Eucalyptus globules Labill* (Myrtaceae) is a kind of medicinal plant.

Its leaves, roots and fruits have been used as traditional remedies for the treatment of influenza, Dysentery, enteritis, rheumatalgia and bleeding. It has been well known for the volatile terpenoid constituents of the essential oil the leaves and fruits.[8]

Fig.1 Picture of *Eucalyptus globules* Leaf and Powder

2. LITERATURE REVIEW

2.1. Taxonomical (Scientific) Classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta (Flowering plant)
Class	:	Magnoliopsida (Dicotyledons)
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	Eucalyptus
Species	:	*Eucalyptus globules Labill*[1].

2.2. Geographical Distribution

Eucalyptus globules will grow on a wide range of substrates, but it is especially common and widespread on soils derived from granite and grano-diorite rocks. It is best developed on moderately fertile loams or heavy, well-drained soil. Blue gum does not occur naturally on poorly- drained soils or on strongly-calcareous or alkaline soils. Most occurrences of *E. globules* are found in areas having an annual rainfall of 60-110 cm, and nowhere does it occur naturally with less than 50 cm annual precipitation.

The natural distribution of *E. globules* is confined to Tasmania, Victoria, and New South Wales between latitudes 31 and 43 degrees S. This species is most common in southeastern Tasmania, islands in the Bass Straits, the Ottway Ranges, and Wilson's Promontory in Victoria.

E.globules has been particularly successful in countries with a Mediterranean-type climate but has also grown well at high altitudes in the tropics. It has failed only in temperate zones with severe winters, in tropical zones at low altitudes where the temperatures are uniformly high, and in regions with long, hot dry seasons. The section within the influence of the moderate coast climate is most favorable for its best development. Though its growth is especially thrifty in the northern coast counties and in the coast valleys of Southern California.[9]

Plantations about 500,000 hectar are currently planted with *E. globules* (1% of the country's land surface), yielding 22% of Spain's total wood production.[10] 15 Different species of Eucalyptus have been introduced to Ethiopia from Australia in 1895 by Menilik II, Eucalyptus have been planted all over the Ethiopian central plateau at altitude from 1400 to 3500 m. With rainfall of 700 – 200 mm per year, until 1975, 90,000 hectar were planted in the surrounding of Addis Ababa and other Ethiopian highland cities, 200,000 hectar of *Eucalyptus globules* and Eucalyptus camaldulensis have been planted on farm level plantation, the first with the best survival and fastest growth the latter fitting for drier and lower sites .[11]

2.3. Morphological Description

It is an aromatic tree in the Myrtle Family (Myrtaceae) which commonly attains a height of 150-180 feet and a diameter of 4-7 feet. It has a straight trunk up to two-thirds of its total height and a well-developed crown. The central trunk and tap root are fringed with many lateral stems and roots. The tap root rarely exceeds a length of 10 feet. The light, yellow-brown bark is deciduous. The leaves of the older branches are narrowly lanceolate, often curved, alternate and hung vertically. They are glossy, dark green, thick and leathery. They average in length from 1.5-2 dm. The leaves of the young shoots are ovate, opposite, sessile, and horizontal. They are covered with a grey, waxy bloom which is much thicker on the bottom surface of the leaf. Young stems are squared or winged. The white flowers are solitary in the axils on flattened stalks. They are approximately 4 - 5.5 cm wide. Flowers are most abundant from December to May. The fruit is a hard, woody capsule, broadly top shaped or globose with a wide flat disc. The fruit is 2 - 2.5 cm across.

The numerous seeds are approximately 2 x 1 mm (relatively small compared to other woody plant species). Seeds are dark brown with a brownish red chaff. The distinctive features of *E. globules* are the juvenile leaves, which are ovate, opposite, sessile, glaucous and occur on squared stems, and the large, solitary fruits (2 - 2.5 cm), which are warty, glaucous and four-ribbed.[12]

2.4. Biology-Ecology

2.4.1. Seeds and Germination

The seeds of the Eucalyptus are very small in comparison with other forest tree seeds but are produced abundantly. *Eucalyptus globules* seeds are among some of the larger seeds in the entire genus (2 x 1 mm). They are formed in a fruit 12 mm in diameter. The mean number of viable seeds produced per 10 grams of total seed is 735 (plus or minus a standard deviation of 380). Most seed is distributed by wind and gravity, but some is moved by such agents as flood, erosion and birds. Under optimal conditions (greenhouse environment at 25 ^{0}C), germination occurs between 5 and 14 days.

In the field, germination should occur no later than 26 days once the appropriate environmental conditions are met. However, seed may remain dormant for several years under dry conditions. *E.globules* is a species which does not require light for the mature seed to germinate. It is difficult for blue gum seeds to germinate within a dense forest of parent trees. *Eucalyptus globules* has a higher percent germination rate in open land.

2.4.2. Pollination

Eucalyptus flowers are mainly pollinated by insects, but birds and small mammals may also act as pollinating agents. There is no evidence that wind plays anything but a minor role in eucalypt pollination. The flowers of Eucalyptus are not highly specialized for insect pollination. The absence of specialization is reflected by the great variety of visiting insects, e.g, Coleoptera, Hymenoptera, Lepidoptera, Hemiptera, and Diptera of which the honey bee is usually regarded as the most important. There is no self-pollination of an individual flower.[10]

2.4.3. Development

Eucalyptus globules is one of the fastest growing eucalyptus. A three-year-old tree has been recorded at 14 m high and 22.5 cm in diameter. At six to eight years, it has been recorded at 25-33 m high, and at 30 years, it has been recorded at 50 m high and 1-2 m in diameter. It has been known to seed as early as its fifth year. An interesting characteristic of development of *E. globules* is the change of its leaves over time.

The extraordinary differences between the young and mature foliage, specifically the difference in position could be protection against transpiration. One of the most characteristic features of the Eucalyptus is the presence of oil glands in the leaves of all species. Most Eucalyptus biologists advocate the theory that the oils protect the leaf against water loss.

2.4.4. Roots

The root system of *E. globules* consists mostly of strong lateral roots. An abundant supply of moisture is demanded. Since the roots grow quickly toward water, *E. globules* should never be planted near wells, cisterns, water pipes, irrigation ditches, sandy or gravelly soils. Large roots have been discovered at a depth of 45 feet below the surface, and surface roots frequently spread over 100 feet away from the trunk.

2.4.5. Drought Resistance

Most eucalyptus grow in localities where there is marked water shortage for substantial parts of the year. Therefore, they are adapted to seasonal drought stress associated with dry summers. They do not economize in the use of water but have wide-ranging root systems and an ability to extract water from the soil at even higher soil moisture tensions than most mesophytic plants. Transpiration rates remain high even when water supply from the soil is dwindling.

It is only when severe permanent wilting occurs that there is stomatal closure which inhibits water loss (and, of course, also prevents gas exchange and photosynthesis) and enables the plant to survive a critical water balance situation for some time.[10]

2.5. *Eucalyptus globules Labill* Plant

Eucalyptus species (Family-Myrtaceae) are remarkable for their rapid growth. Some species of them, in their natural habitat, attain gigantic size and are among the tallest trees of the world.[12] Most of the species are popularly called "gum trees" although the exudation from them is not a gum, but an astringent.[13] Content: minimum 20 ml/kg of essential oil for the whole drug (anhydrous drug) and minimum 15 ml/kg of essential oil for the cut drug (anhydrous drug).

It contains a minimum of 2% V/m essential oil. It is cultivated in many parts of the world. Eucalyptus is an evergreen tree with leathery leaves. There are several varieties of Eucalyptus. *Eucalyptus globules* is the most popular in terms of cultivation and medicinal use.[14]

2.6. Essential Oils

2.6.1. General

Essential oils are concentrated volatile, natural, highly concentrated, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. These oils are extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total.[15] Essential oils are usually obtained by steam or hydro-distillation first developed in the middle ages by arabs.[16]

2.6.2. Chemical Constituents of Essential Oils

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups

Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

Non volatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids. Classes of essential oil compounds and their biological activities are as follows.

2.6.2.1. Hydrocarbons

The majority of essential oils fall into this category; these contain molecules of hydrogen and carbon only and are classified into terpenes (monoterpenes: C_{10}, sesquiterpenes: C_{15}, and diterpenes: C_{20}). These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic or tricyclic) or aromatic. Limonene, myrcene, p-menthane, alpha - pinene, p-cymene, myrcene, alpha – phellandrene, thujane, fenchane, farnesene, azulene, cadinene and sabinene are some examples of this family of products. Some structures of these compounds are given in (Figure 2).[17]

2.6.2.2. Esters

Esters are sweet smelling and give a pleasant smell to the oils and are very commonly found in a large number of essential oils. They include for example, linalyl acetate, geraniol acetate, eugenol acetate and bornyl acetate (Figure 3).

2.6.2.3. Oxides

Oxides or cyclic ethers are the strongest odorants, as it is the most omnipresent one inessential oils. Examples of oxides are bisabolone oxide, linalool oxide, sclareol oxide and ascaridole (Figure 4). Their therapeutic benefits are expectorant and stimulant of nervous system

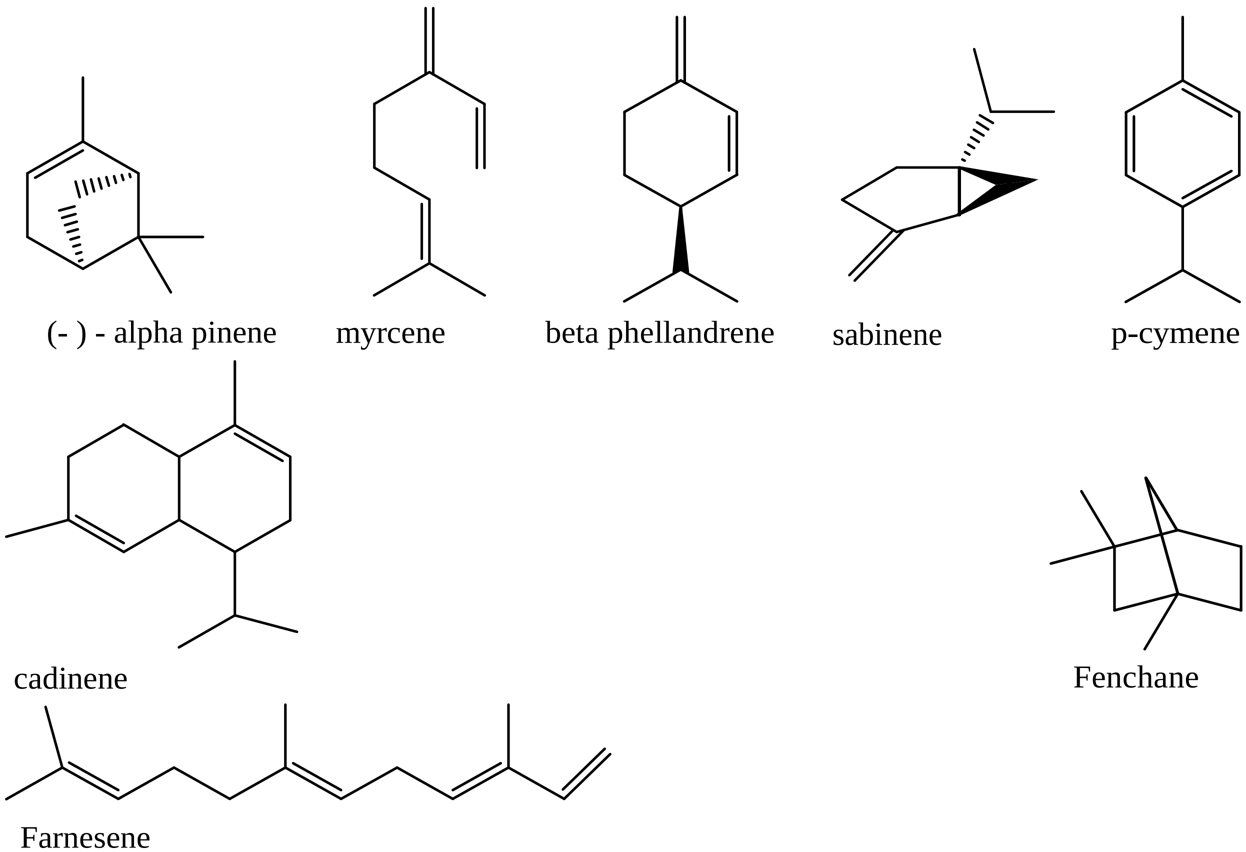


Fig. 2 Structure of some hydrocarbons commonly found in essential oils

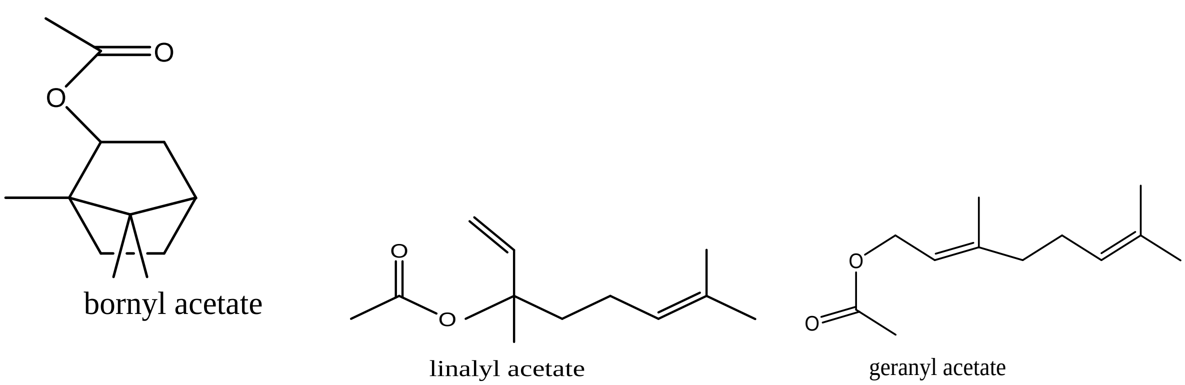
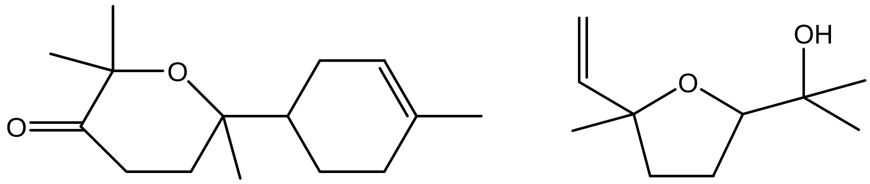


Fig. 3 Structure of some esters commonly found in essential oils

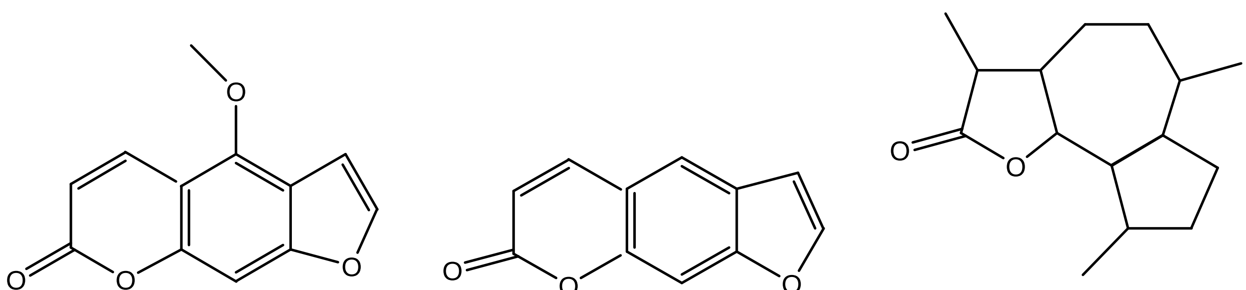


bisabolone oxide linalool oxide

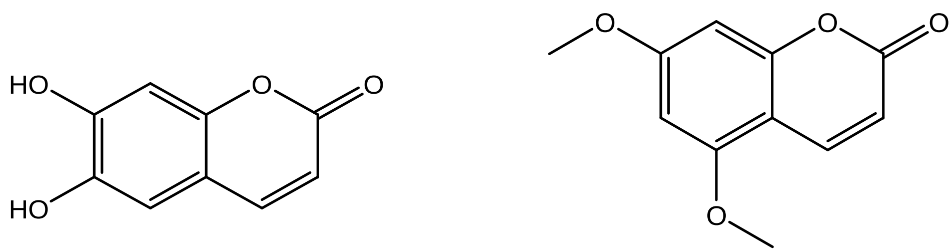
Fig. 4 Structure of some oxides commonly found in essential oils

2.6.2.4. Lactones

Lactones are of relatively high molecular weight and are usually found in pressed oils. Some examples of lactones are nepetalactone, bergaptene, costuslactone, dihydronepetalactone, alantrolactone, epinepetalactone, aesculatine, citroptene, and psoralen (Figure 5). [17]



bergaptene psoralen Costuslactone



Asculalinc Eltroplene

Fig. 5 Structure of some lactones commonly found in essential oils

2.6.2.5. Alcohols

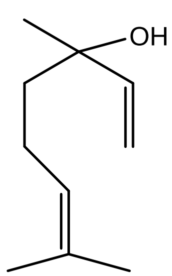
In addition to their pleasant fragrance, alcohols are the most therapeutically beneficial of essential oil components with no reported contraindications. They are antimicrobial, Tantiseptic,tonifying, balancing and spasmolytic. Examples of essential oil alcohols are linalol, menthol, borneol, santalol, nerol, citronellol and geraniol (Figure 6)

2.6.2.6. Phenols

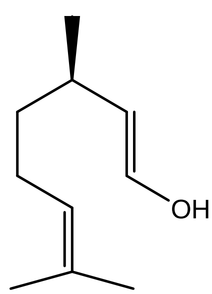
These aromatic components are among the most reactive, potentially toxic and irritant, especially for the skin and the mucous membranes. Their properties are similar to alcohols but more pronounced. They possess antimicrobial, rubefacient properties, stimulate the immune and nervous systems and may reduce cholesterol. Phenols are often found as crystals at room temperature, and the most common ones are thymol, eugenol, carvacrol and chavicol (Figure 7).

2.6.2.7. Aldehydes

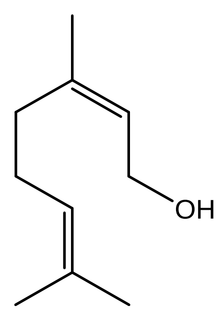
Aldehydes are common essential oil components that are unstable and oxidize easily. They have characteristically sweet, pleasant fruity odors and are found in some of our most well known culinary herbs such as cumin and cinnamon. Common examples of aldehydes in essential oils include citral (geranial and neral), myrtenal, cuminaldehyde, citronellal, cinnamaldehyde and benzaldehyde (Figure 8).[17]



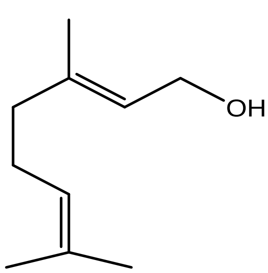
linalool



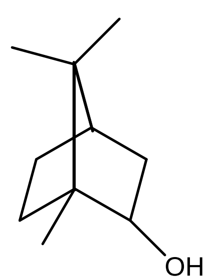
Citronellol



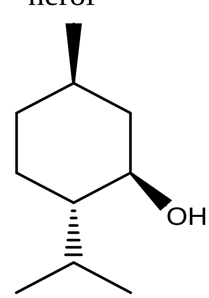
nerol



Geraniol

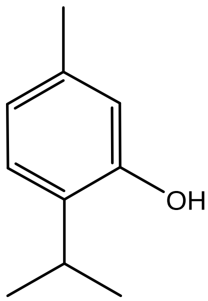


Borneol

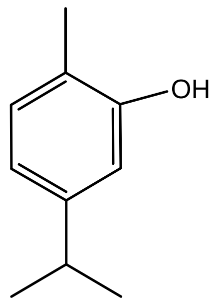


Menthol

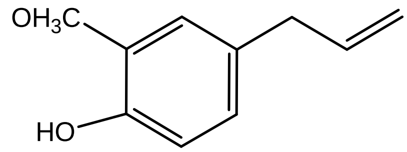
Fig. 6 Structure of some alcohols commonly found in essential oils



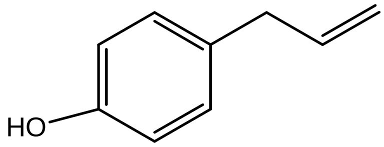
Thymol



Carvacrol

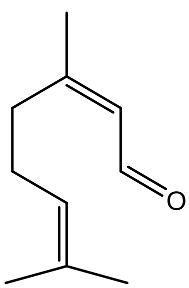


Eugenol

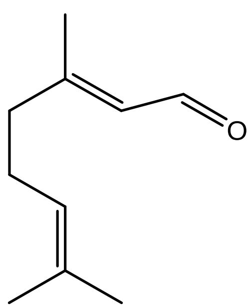


Chavicol

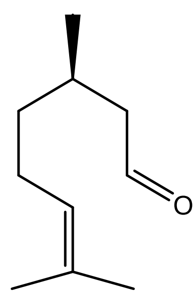
Fig. 7 Structure of some phenols commonly found in essential oils



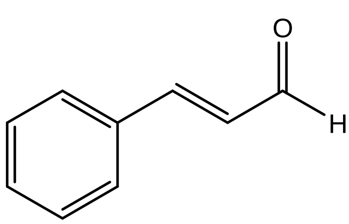
Neral



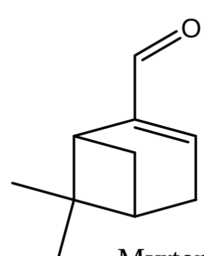
Geranial



Citronellal



Cinnamaldehyde

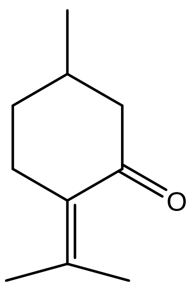


Myrtenal

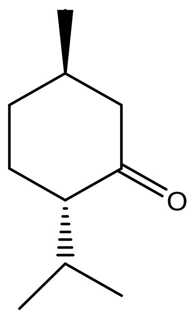
Fig. 8 Structure of some aldehydes commonly found in essential oils

2.6.2.8. Ketones

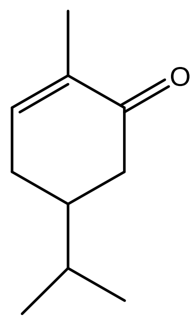
Ketones are not very common in the majority of essential oils; they are relatively stable molecules and are not particularly important as fragrances or flavor substances. In some cases, ketones are neurotoxic and abortifacients such as camphor and thujone but have some therapeutic effects. Due to their stability, ketones are not easily metabolized by the liver. Common examples of ketones found in essential oils include carvone, menthone, pulegone, fenchone, camphor, thujone and verbenone (Figure 9).[17]



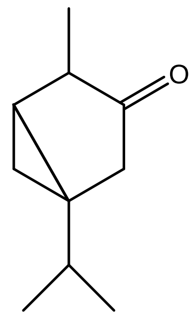
Pulegone



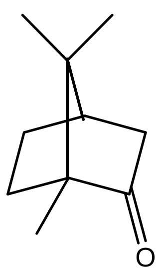
Menthone



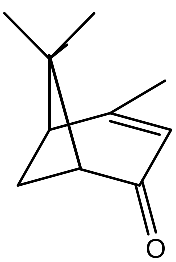
Carvone



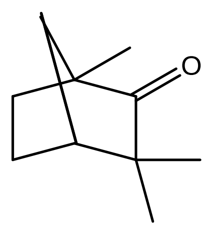
Thujone



Camphor



Verbenone



Fenchone

Fig. 9 Structure of some ketones commonly encountered in essential oils

2.7. Eucalyptus Oil

2.7.1. General

A few Eucalyptus species, produce a leaf oil for which there is an existing limited world trade as a pharmaceutical product and a large potential market as an industrial solvent. These oils are composed of mixtures of volatile organic compounds including hydrocarbons, alcohols, aldehydes, ketones, acids, ethers and esters. Most are monoterpenes and sesquiterpenes which consist of two or more isoprene (C_5H_8) units. They are products of photosynthesis, with functions for the plants that are still poorly understood. Eucalyptus oil has numerous traditional uses, especially in non-prescription pharmaceuticals, but the market is small.

Eucalyptus oil based products have been used as a traditional non-ingestive treatment for coughs and colds. It has uses as a fragrance in soaps, detergents and perfumes and as a flavouring in food. Household uses include spot and stain remover and wool wash component. It has also been used as a flotation agent in the mining industry.[15]

All Eucalyptus oils are renowned for their antiseptic qualities, while some species have been shown to inhibit viruses as well. Oils high in terpene hydrocarbons are considered the most effective against influenza viruses.[18]

2.7.2. Physical and Chemical Properties of Eucalyptus Oil

2.7.2.1. Camphene

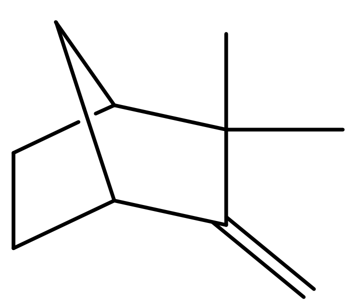


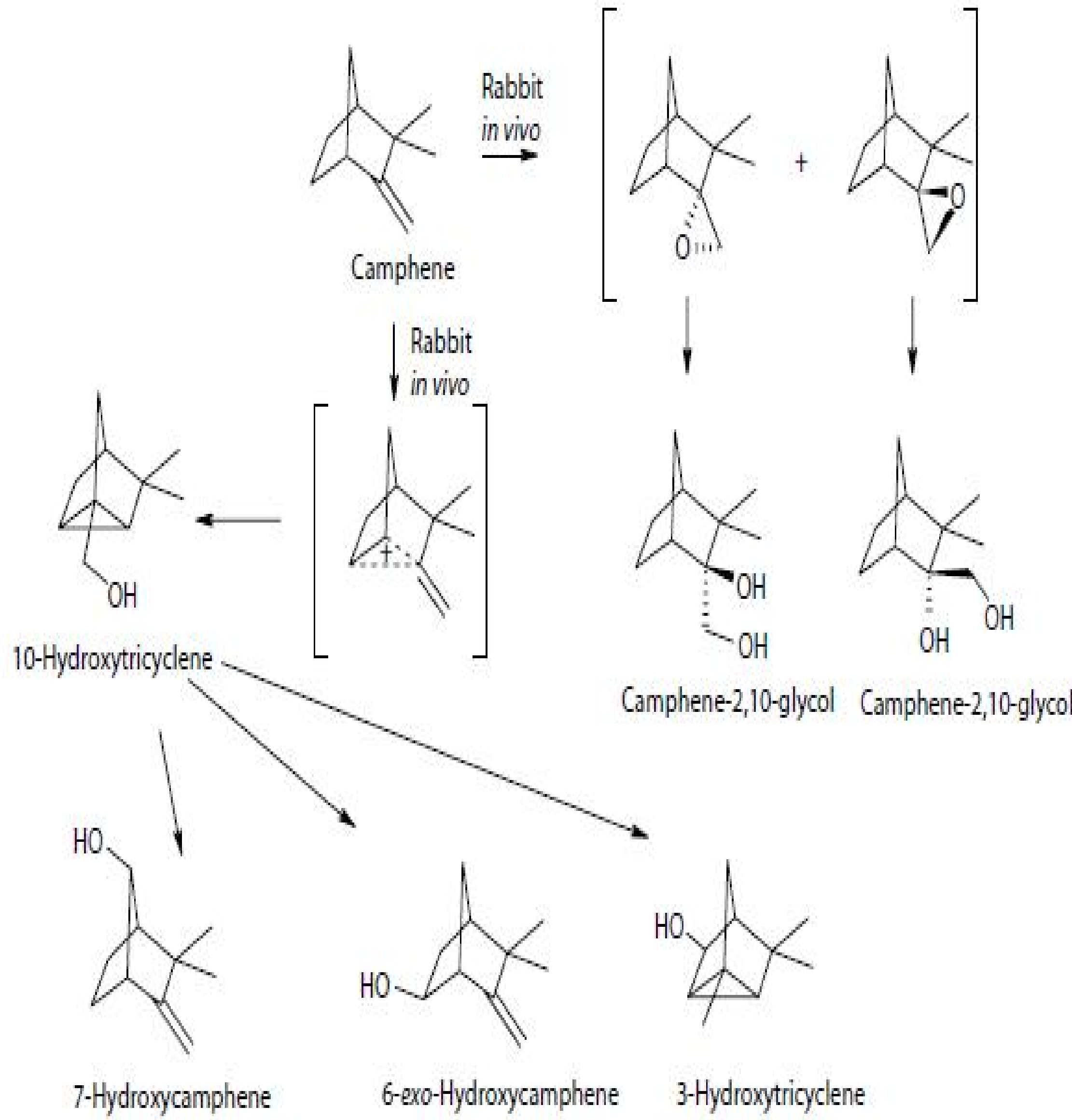
Fig. 10 Structure of Camphene (2, 2-dimethyl-3-methylene – bicycle [2.2.1]heptane)

Camphene is the only well - recognised terpene which occurs in nature in the solid condition. Camphene is extremely difficult to separate in the solid condition from essential oils, and it may therefore be taken for granted that natural camphene is rarely prepared in the pure condition. The figure given above for the specific rotation is for a sample artificially prepared from pinene hydrochloride.

Camphene is prepared artificially by the isomerisation of pinene with sulphuric acid or by the withdrawal of HC1 from pinene monohydrochloride, or by the action of heat in the presence of acetic anhydride on bornylamine, $C_{10}H_{17}NH_2$, which causes the withdrawal of ammonia and leaves camphene, as follows

$C_{10}H_{17}NH_2 \rightarrow C_{10}H_{10} + NH_3$

Camphene is not very stable at high temperatures, and when kept at 250 °C decomposes to a considerable extent, yielding other terpenes.[20] Camphene is found in many plants at high concentrations. Data about the *in vivo* metabolism of camphene are scarce as there is only one publication demonstrating various biotransformation products in the urine of rabbits after its oral administration. As shown in Scheme 1, camphene is metabolized into two diasteromeric glycols (camphene-2, 10-glycols) IUPAC name 2, 2 dimethy l- 3 - methylene - bicyclo [2.2.1] heptan.
Their formation obviously involves two isomeric epoxide intermediates, which are hydrated by epoxide hydrolase. Further metabolites, namely 6-hydroxycamphene, 7-hydroxycamphene,
3-hydroxytricyclene, and 10-hydroxytricyclene, were apparently formed through the non classical cation intermediate.[19]



Scheme .1 Urinary excretion of Camphene metabolites in rabbits

2.7.2.2 Pinene

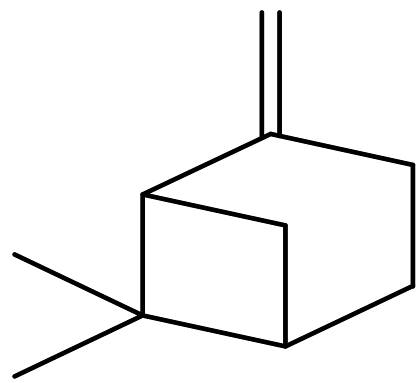


Fig. 11 Structure of pinene (6, 6 – dimethyl – 2 – methylenebicyclo [3.1.1] heptane)

There are four distinct terpenes known under this name namely, alpha pinene, beta pinene, delta pinene and isopinene. It must be emphasized. However, that they are all of different constitution. Alpha pinene is the most commonly occurring terpene found in nature. Alpha - pinene is found in the essential oil of the racemic mixture in eucalyptus oil.[19] Alpha - pinene forms a number of well-defined crystalline compounds, several of which serve for its identification. Of these one of the best known is the nitrosochloride.

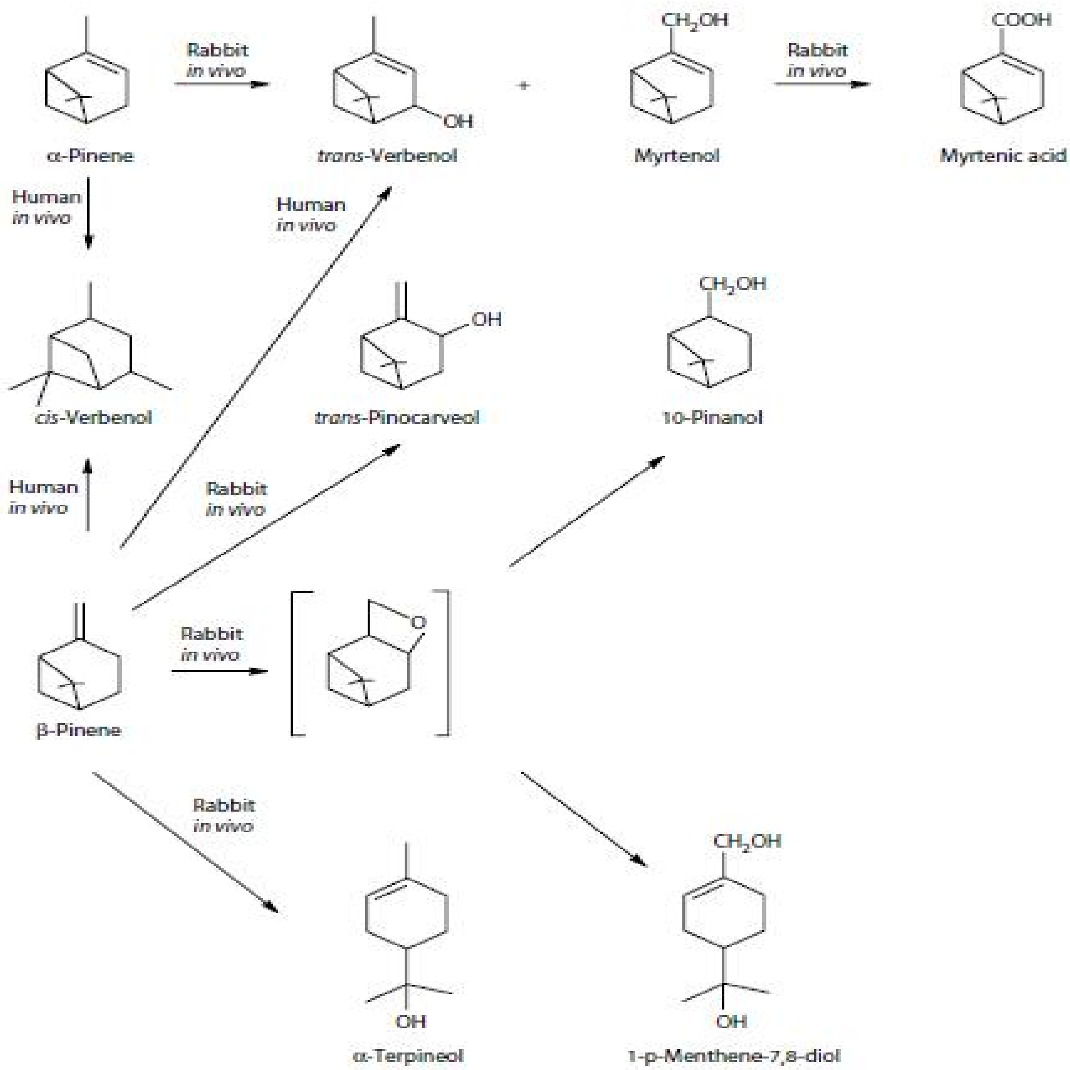
The formula for pinene - nitrosochloride has been assumed to be $C_{10}H_{16}NOCl$ but Baeyer showed it to be a bi-molecular compound of the formula $(C_{10}H_{16}NOC1)_2$, Nitroso-pinene is obtained from pinene - nitrosochloride by the action of alcoholic potash

$C_{10}H_{16}NOC1 + KOH = C_{10}H_{15}NO + KC1 + H_2O$.

An important compound for the identification of pinene is the hydrochloride $C_{10}H_{16}HC1$, a body once known as artificial camphor, on account of its odour being very similar to that of natural camphor. Pinene hydrochloride is a volatile substance, having a camphor-like odour, and melts at 127 °C.[20] As shown in Scheme 2, metabolism of alpha - and beta - pinene in humans leads to the formation of *trans*- and *cis*- verbenol, respectively.

Recent data analyzing the human urine after occupational exposure of sawing fumes also suggest that *cis* and *trans*-verbenol are being further hydroxylated to diols.

The main urinary metabolite of alpha - pinene in rabbits is *trans*-verbenol; the minor biotransformation products are myrtenol and myrtenic acid. The main urinary metabolites of beta pinene, in rabbits, however, is *cis* verbenol indicating stereoselective hydroxylation.[19]



Scheme. 2 proposed metabolism of alpha and beta pinene in rabbits and humans

2.7.2.3. Terpineol

Alpha -Terpineol, a monocyclic monoterpene tertiary alcohol. After oral administration to rats (600 mg/kg body weight), alpha - terpineol is metabolized to *p*-menthane - 1, 2, 8 - triol probably formed from the epoxide intermediate. Notably, allylic methyl oxidation and the reduction of the 1, 2 - double bond are the major routes for the biotransformation of alpha-terpineol in rat Scheme 3. Although allylic oxidation of C-1 methyl seems to be the major pathway, the alcohol *p*-ment-1- ene - 7, 8 - diol could not be isolated from the urine samples. Probably, this compound is accumulated and is readily further oxidized to oleuropeic acid.[19]

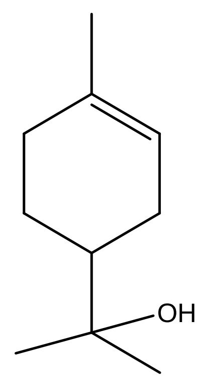
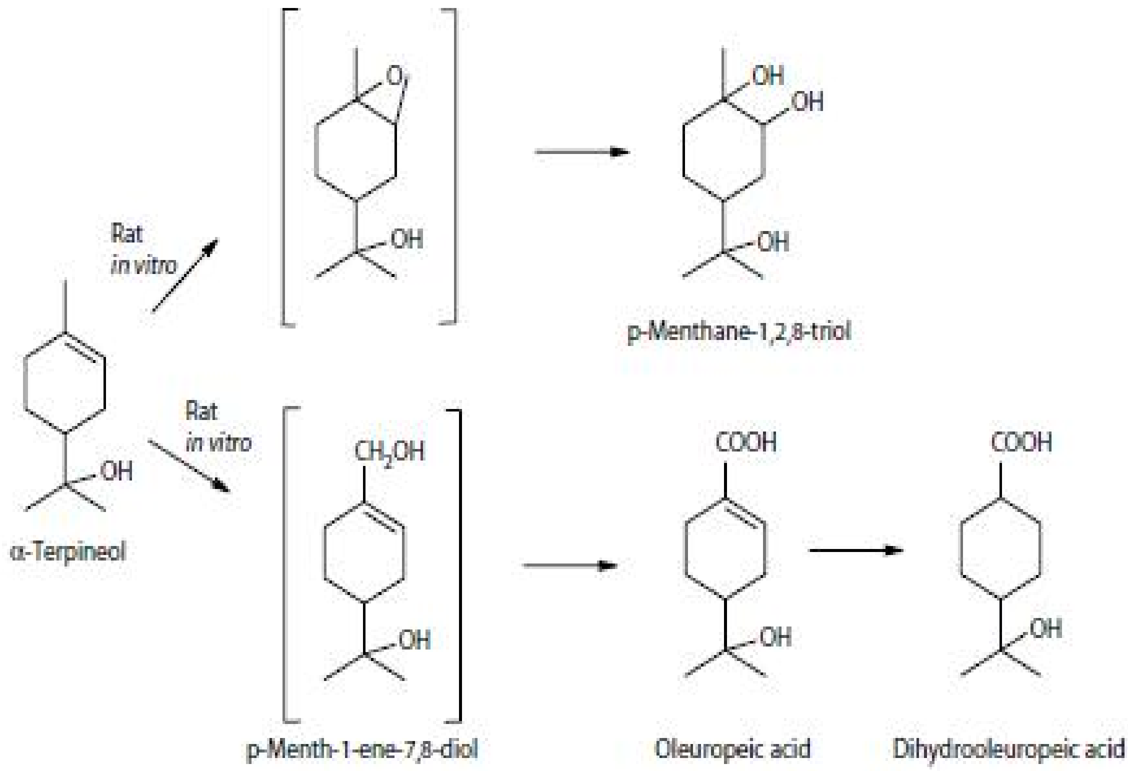


Fig.12 Structure of alpha Terpineol 2 - (4 – methyl -1- cyclohex – 3 - ene) propan – 2 - ol



Scheme 3. Proposed metabolism of alpha terpineol in rats

Terpineol $C_{10}H_{17}$. OH, is an alcohol of the greatest interest from a scientific point of view, and of the highest practical importance from the perfumer's point of view. Three well-defined modifications of the substance known as terpineol are recognised, These " terpineols " are known as alpha - terpineol, beta-terpineol, and gama-terpineol.

Terpineol of commerce is, in the main, a mixture of the isomers, in which alpha - terpineol largely predominates. Gama - terpineol has not been found in nature. It has been prepared by the reduction of tri-bromo – [1.4.8] - terpene, resulting from the bromination of dipentene dihydrobromide. It also results from the action of dilute phosphoric, or oxalic acid, on terpin hydrate.[20]

2.7.2.4. Myrcene

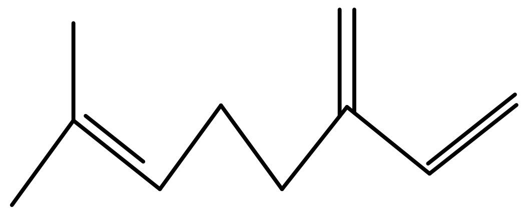
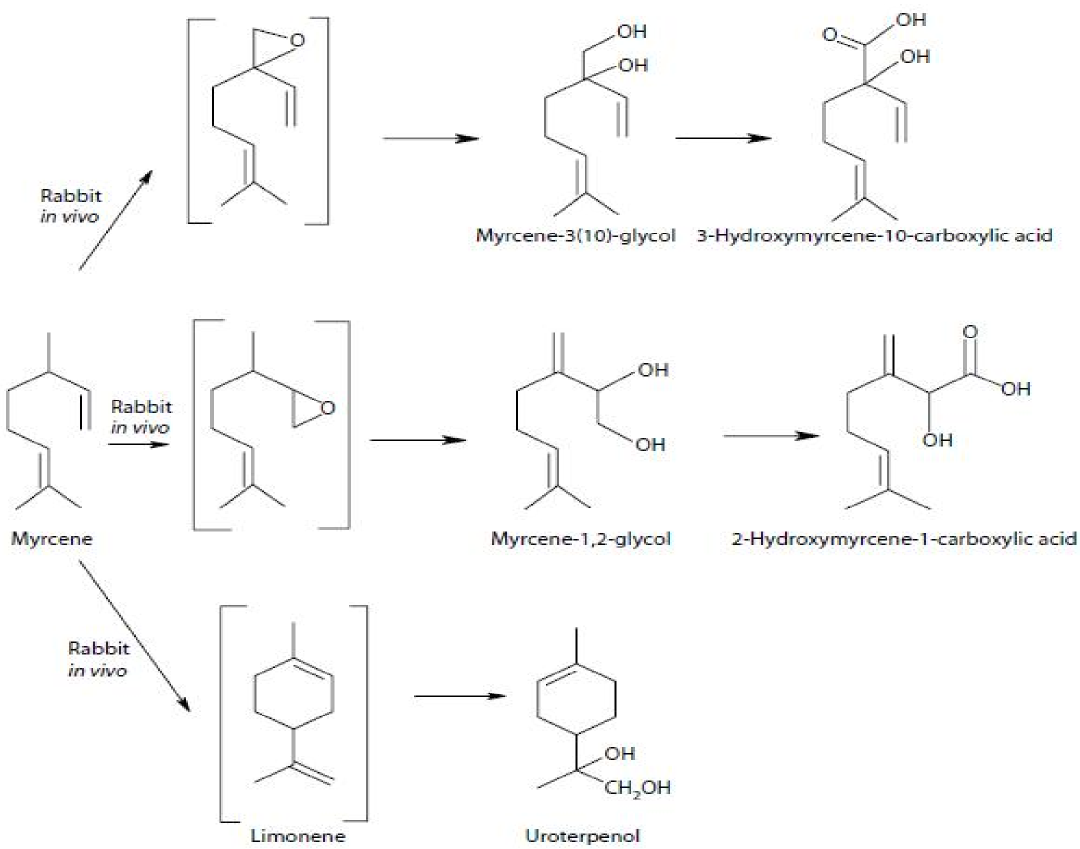


Fig.13 Structure of Myrcene (7 – methyl – 3 – methylene -1, 6-octadiene)

After oral application, can be identify several metabolites in the urine of rabbits whereby the formation of the two glycols may be due to the hydration of the corresponding epoxides formed as intermediates. The formation of uroterpenol may proceed through limonene, which was clearly derived from myrcene in the acidic conditions of rabbit stomachs Scheme 4.[19]



Scheme.4 Proposed metabolism of myrcene in rabbits

Myrcene has been found in a number of essential oils, such as bay oil, West Indian lemon-grass oil, the oil of *Lippia citriodora, Eucalyptus* etc. It has sufficient resemblance to the terpenes proper to have been classified as an "olefinic" terpene. Its constitution being that of an open chain, and not a ring compound.[20]

2.7.2.5. Ocimene

This body is very closely related to myrcene, and has been found in the oil of basil distilled in Java, Eucalyptus etc.[20]

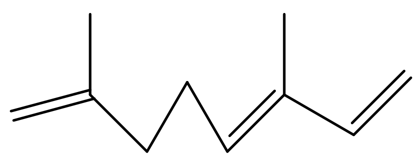


Fig.14 Structure of Ocimene (cis -3, 7 – dimethyl-1, 3, 7 - octatriene)

2.7.2.6. Globulol

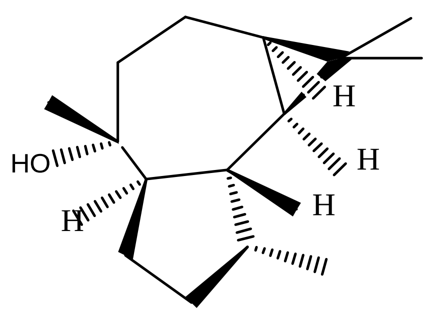


Fig.15 Structure of Globulol

This sesquiterpene alcohol was discovered by Schimmel & Co. in oil of *Eucalyptus globulus*. It is found in the last fractions of the distillate, separating out in crystalline condition. On recrystallization from 70 percent, alcohol, it was obtained in the form of brilliant, almost odorless needles, its formula is $C_{15}H_{26}O$.[20]

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2.7.2.7. Aromadendrene

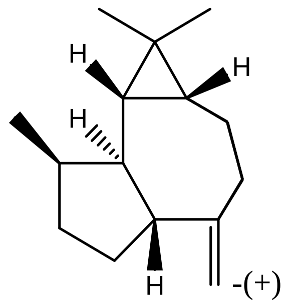

Fig. 16 Structure of Aromadenderene

This aldehyde has been isolated from various *Eucalyptus* oils. It has a pleasant odour resembling that of cumic aldehyde. This, however, is improbable and it to have the formula $C_9H_{12}O$, which would make it to be a lower hornologue of the terpenic aldehydes. Its physical characters, however, are somewhat doubtful, as specimens isolated from the oils of *Eucalyptus hemiphloia* and *Eucalyptus salubris* show.[19]

3. OBJECTIVE OF THE STUDY

3.1. General Objective

The objective of this study is to isolate and characterize the major component of the leaf oil of *Eucalyptus globules* plant.

3.2. Specific Objective

- To determine a working procedure for the isolation of the major component of the leaf oil of *Eucalyptus globules*

- To characterize the structures of the major component of the leaf oil of *Eucalyptus globules* plant using spectroscopic methods, such as IR, NMR, etc.

4. EXPERIMENTAL

4.1. Instrument and Apparatus

Electronic balance, hydro distillation apparatus, grinder, roundbottom flask, conical flask, beaker, stand, Bunsen burner, separatory funnel, Whatman filterpaper, Buchner funnel, graduated cylinder, condenser, TLC paper, capillary tube, water pump, oven, IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 Spectrometers.

4.2. Reagents and Chemicals

Analytical grade chemicals, reagents and distilled water, petroleum ether, chloroform, methanol, solid iodine, boiling chips, unhydrous Na_2SO_4, conc H_2SO_4, HCl, KI, NaCl, $FeCl_3$, were used.

.

4.3. Sample Collection and Preparation

Fresh leaves of *Eucalyptus globules* trees were collected intightly closed plastic bags in October, 2014 from Amhara region South Gondar. The botanical identification of the material collected was carried out by Gondar University Biology departement, Ethiopia and voucher specimens were deposited at room temperature in an airtight container until used for the extraction.

4.4. Methods of Extraction and Isolation

4.4.1. Extraction and Isolation

The leaves of *E.globules* were air dried at room temperature and were finely powdered in an electrical grinder. About 150 g of leaf powder and 900 ml distilled water subjected to hydro distilled for 3 hours in a hydro distillation apparatus for extraction of the essential oils. The oil and water mixture were collected in a 100 ml conical flask. The mixture were removed every one hour from the conical flask to decrease evaporation of the oil due to heat. As the oil and water mixture were found to be nearly immiscible, the mixture were separated using separatory funnel and the oil was collected. The oil extracted from each sample was found containing fractions of water, which was removed by adding small amount of anhydrous sodium sulfate. The oil was filtered, and kept in a bottle. The oils obtained were light yellow, with a strong odor and it was ready for the next subsequent analysis.

4.4.2. Purification

The oil was passed to the fractional distillation process and distilled between 173 °C and 190 °C. And cooled, then checked by TLC. The TLC showed the presence of trace amounts of impurities which were unable to purify them further.

4.5. Phytochemical screening test on the isolated oil

The following phytochemical screening tests were done on the oil using standared methods. The results are shown in table 1 below.

Table1. Phytochemical scrrening test on the isolated oil

Phytochemical Test	Eucalyptus globules oil
Alkaloids	-
Phytosterols	-
Phenols	-
Tannins	-
Flavanoids	-
Saponins	-
Quinons	-
Terpenoids	+

Key, present = + absent = -

5. RESULT AND DISCUSSION

5.1. Characterization of Compound Ayu-A

Characterization of the compound was done by using chemical test and spectroscopic techniques such as, IR, ^{1}H NMR, ^{13}C NMR and DEPT. The chemical test indicated the presence of terpenoids.

5.2. Spectroscopic Analysis

5.2.1. The IR Data of Compound Ayu-A

The IR spectrum (Apendix 1) showed the band at 2928 cm^{-1} due to sp^3 C-H stretching. The bands at 1377 and 1446 are due to CH_3 and CH_2 bending absorption respectively. The band showed at 1080 is due to the C-O Stretch .The band at 3500 cm^{-1} could arose from the presence of water as impurity. The IR data of compound Ayu-A and the normal ranges are given in table 2 below.

Table 2 IR data of compound Ayu-A

Major band	Value compound Ayu-A in cm^{-1}	Expected values (from table) in cm^{-1}
SP^3 C-H stretch	2928	3000-2840
CH_2 bending	1446	1465
CH_3 bending	1377	1375
C-O stretch	1080	1300-1000

5.2.2. The ^{1}H NMR Chemical Shift of Compound Ayu-A

From the ^{1}H NMR spectrum (appendix 2) only the major signals were taken for the structural elucidation of compound Ayu-A and others were considered as impurities. Accordingly the tall singlet peak at 1.1 ppm is due to protons of methyl groups. The signals at 1.2, 1.29, 1.49 and 1.55 ppm are assigned for the protons of the methylene groups. Finally the peak at 1.7 ppm is assigned for the methine proton. The proton ^{1}H NMR chemical shifts of compound Ayu-A and the suggested structure from literature are summarized in table 3 below. The ^{1}H NMR chemical shiefts of the specific protons of suggested structure are also shown in figure 17 below.

Table 3 ^{1}H NMR Chemical shifts of compound Ayu-A and suggested structure from litrature

Node	Chemical shifts of compound Ayu-A in ppm	Chemical shifts of suggested structure from litrature in ppm
C-H	1.7	1.74
CH_2	1.2	1.27
CH_2	1.49	1.52
CH_2	1.55	1.65
CH_2	1.29	1.40
CH_3	1.1	1.26
CH_3	1.1	1.26
CH_3	1.1	1.31

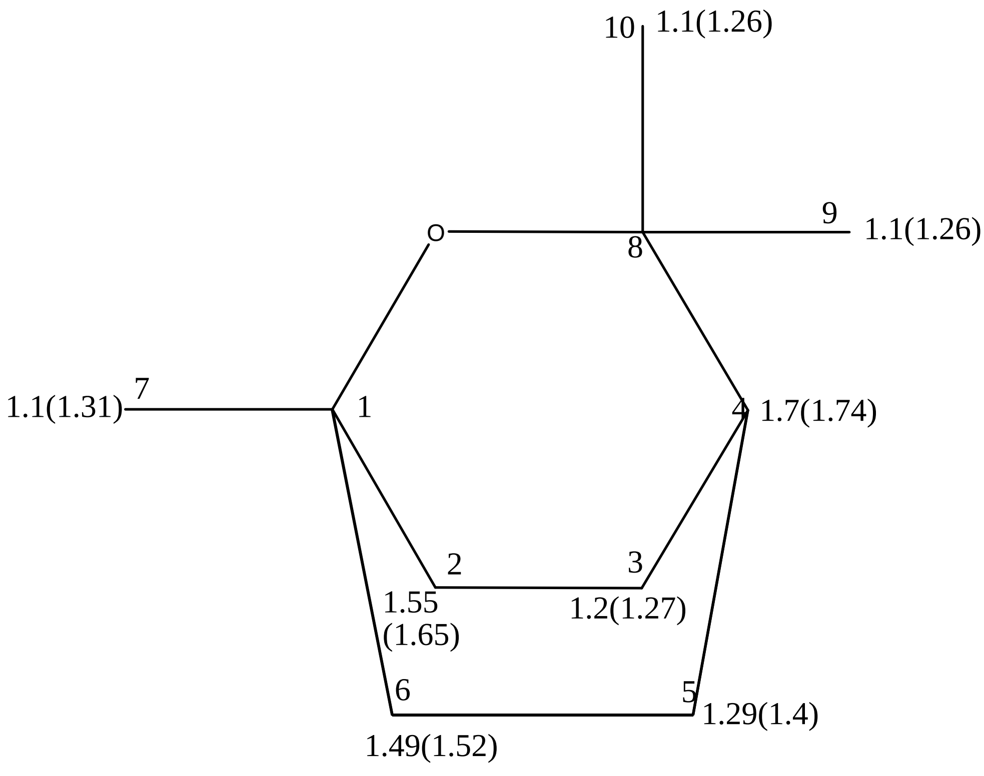

Fig.17 Sugested structure of compound Ayu-A (1, 3, 3 – Trimethyl – 2 - Oxabicyclo [2.2.2] – octane) and chemical shifts of the specific protons (the chemical shifts in parenthesis are values obtained from literature).

5.2.3. ^{13}C NMR and DEPT Chemical Shifts of Compound Ayu-A

The proton decoupled ^{13}C NMR and DEPT spectra of compound Ayu-A (Appendix 3 and 4) showed the presence of Methyl, Methylene, Methine and quaternary carbon atoms, By examining the chemical shifts and peak intensities the taller peak at 28.67 ppm could arise from carbons 9 and 10 which have three attached hydrogens each. The next taller peak at 27.86 ppm must arise from carbon 7 which has three attached hydrogens. By referring the DEPT-135 spectrum the peak at 41.03 ppm, is positive while the peaks at 35.24 and 24.73 ppm are negative. Considering the knowledge of dishielding effects of electronegative elements the peak at 41.03 ppm could be assigned to carbon 4. Similarly the peak at 35.24 ppm to carbons 2 and 6 and the peak at 24.73 ppm to carbons 3 and 5 could be assigned. Finally the peaks at 69.78 and 73.63ppm could arise from carbons 1 and 8 respectively which have no attached hydrogens.

The ^{13}C NMR and DEPT chemical shifts of compound Ayu-A as well as suggested structure from literature are shown in table 4 below.

Table 4. ^{13}C NMR and DEPT Chemical shifts of compound Ayu-A at suggested structure from literature

Carbon number	^{13}C NMR chemical shift of compound Ayu-A	^{13}C NMR chemical shift of suggested structure	DEPT
1	69.78	72.7	Quaternary
2 and 6	35.24	37.4	Two equivalent CH_2
3 and 5	24.73	24.2	Two equivalent CH_2
4	41.03	39.7	CH
7	27.86	25.5	CH_3
8	73.63	76.8	Quaternary
9 and 10	28.67	25.5	Two equivalent CH_3

The chemical test as well as the spectroscopic data suggest that the most probable structure of compound Ayu-A to be 1, 3, 3 – Trimethyl – 2 - Oxabicyclo [2. 2. 2] Octane. As shown in Fig 17 above. This has been compared with the ^{1}H NMR and ^{13}C NMR chemical shifts of 1, 3, 3 -

Trimethyl - 2 - Oxabicyclo [2.2.2] octane. From the literature and were found in agreement. The compound Ayu-A were pale yellow color Liquid with a camphor odor. It was soluble in alcohol, chloroform, methanol, ether but insoluble in water, its boiling point was 176 – 177 ^{o}C.

6. Conclusion and Recommendation

6.1 Conclusion

It can be concluded from the present study that *Eucalyptus globules* is a potential source of essential oils. Several essential oils have been observed from the oil part as checked by TLC. Out of these only the major component was isolated, the most probable structure of this major component is 1, 3, 3, - Trimethyl -2- Oxabicyclo [2.2.2] – octane. Other spectroscopic techniques such as, MS, COSY, HETCOR are necessary for complete structural elucidation of the isolated compound.

6.2 Recommendation

As mentioned above the oil of *Eucalyptus globules* is rich in essential oils. Other components of the oil must be isolated and characterized. In addition to this the oil of *Eucalyptus globules* is medicinally important as noted in the literature. So anti microbial activities on the oil part as well as on the isolated compound shall be performed.

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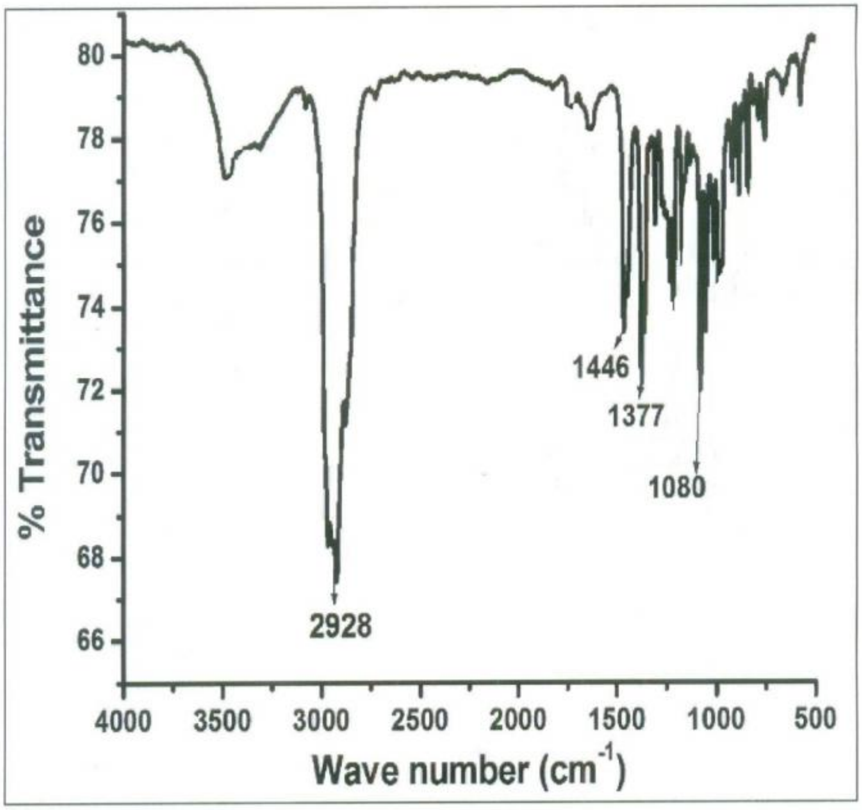
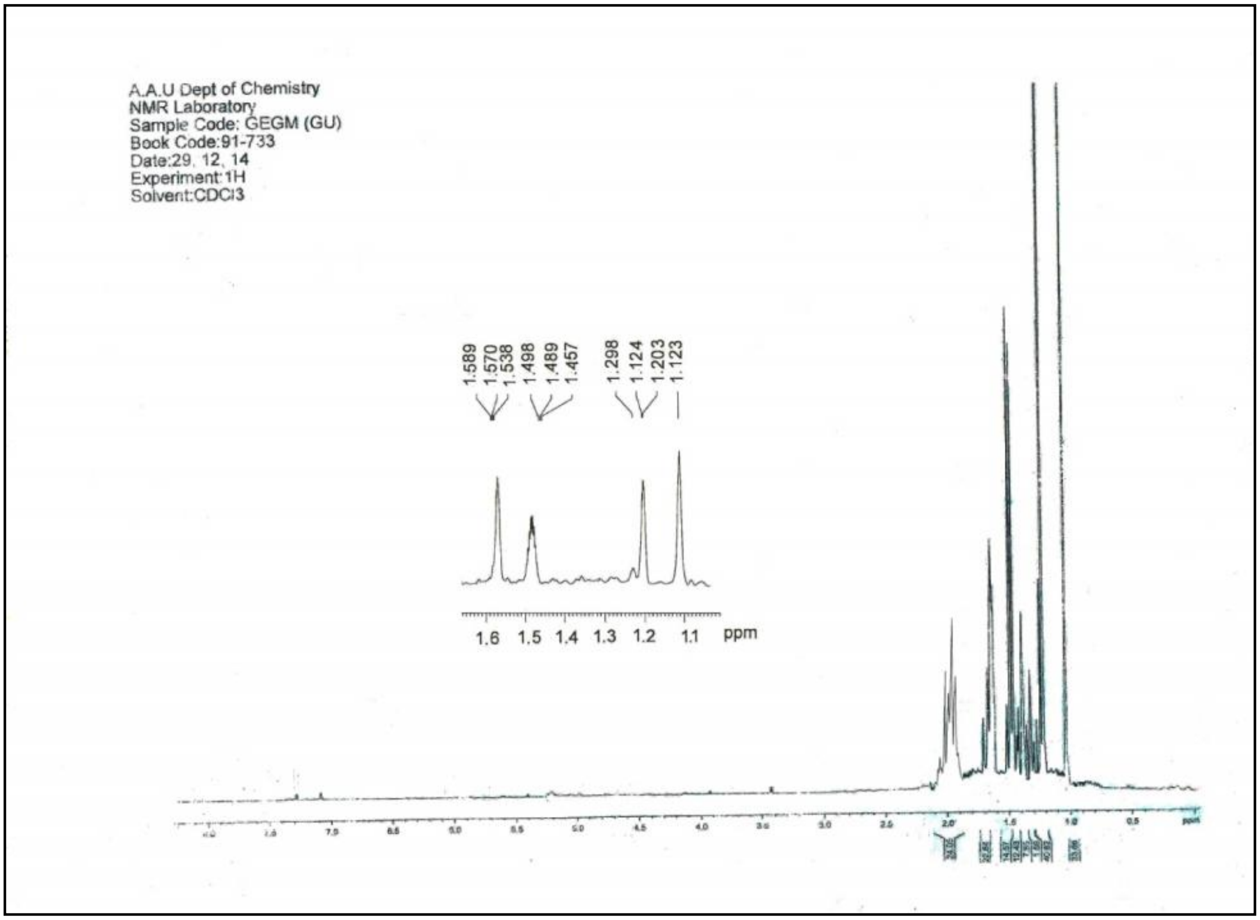
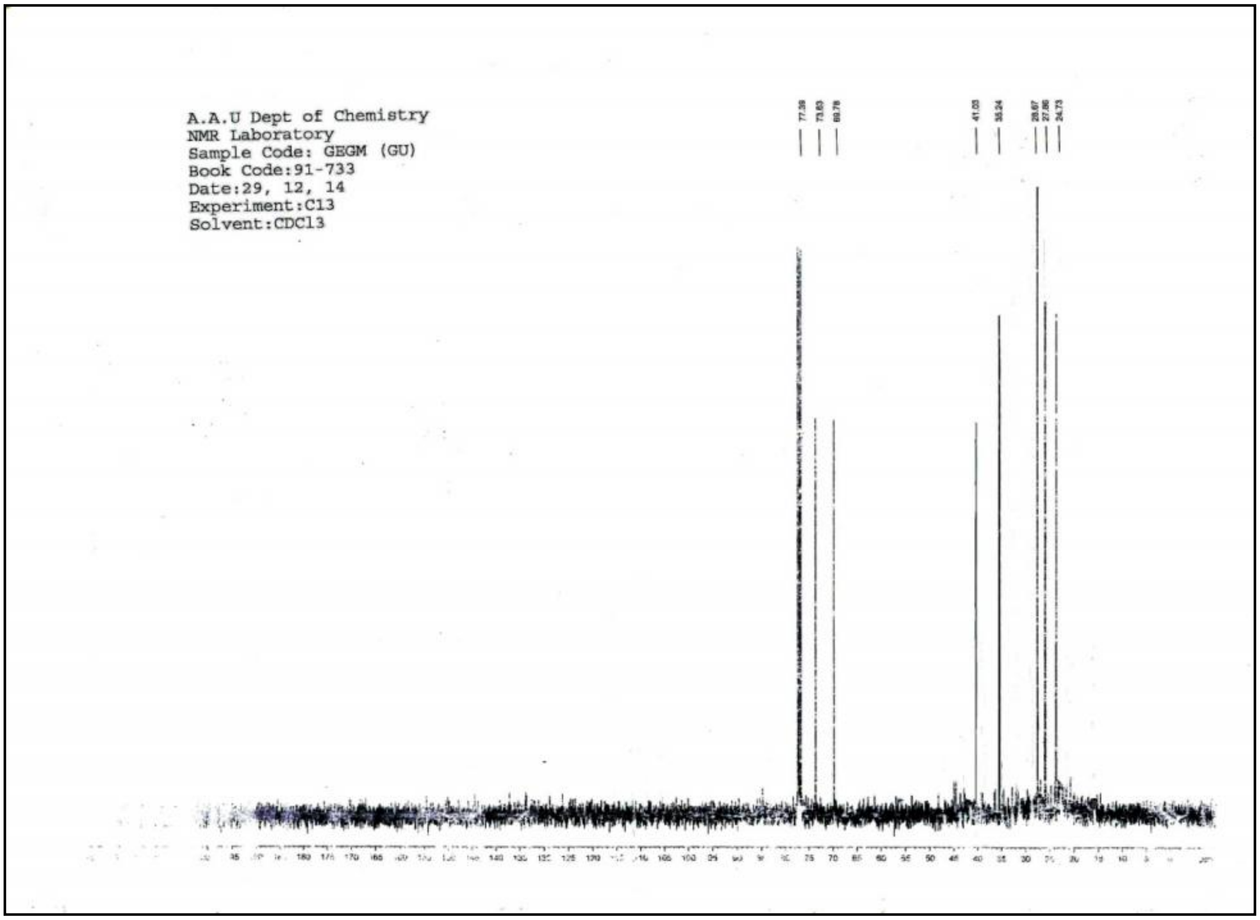


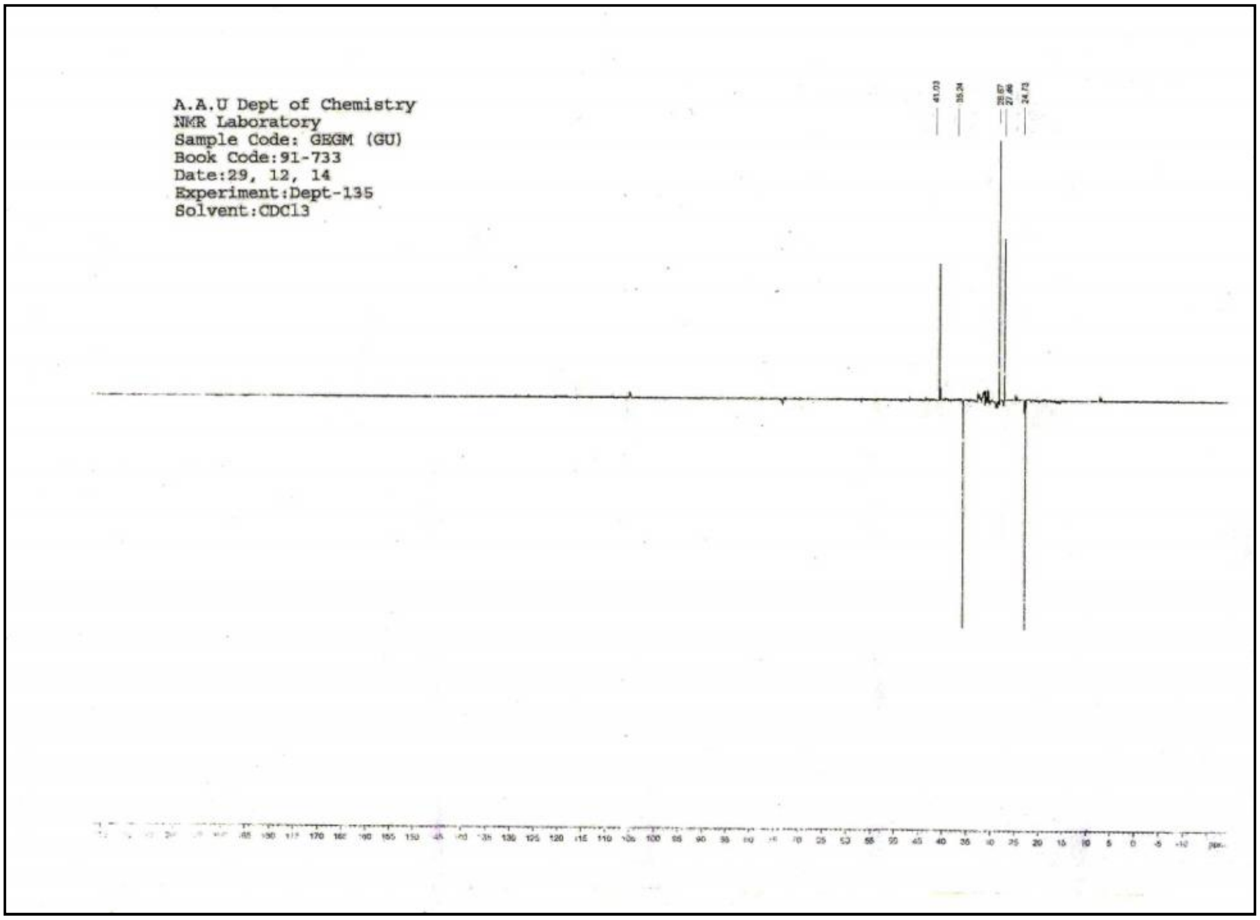
Appendix 1: IR Spectrum of Compound Ayu-A



Appendix 2: ^{1}H NMR Spectrum of Compound Ayu-A



Appendix 3: ^{13}C NMR Spectrum of Compound Ayu-A



Appendix 4: DEPT Spectrum of Compound Ayu-A